UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

November 18, 2015

MEMORANDUM

Subject: Efficacy Review for FruitGard; EPA Reg. No. 79814-5; DB Barcode: D423207.

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Applicant: ICA TriNova, LLC
1 Beavers St., Suite B
Newnan, GA 30263

Formulation from the Label:

Active Ingredients	% by wt.
Sodium Chlorite	3.2 %
Other Ingredients:	96.8 %
Total	100.0 %

I. BACKGROUND

The product FruitGard (EPA Reg. No. 79814-5), is a registered product for use in potato storage facilities to fumigate against non-pathogenic spoilage organisms, such as yeasts and molds. The current data package is submitted to amend the registration of this product to add a new use for post-harvest control of *Salmonella* & *E. coli* O157:H7 on tomatoes and cucurbit crops (Crop Group 9) including cantaloupes. Studies were conducted at BCS Laboratories, Inc., located at 4609 NW 6th St. Bldg. A, Gainesville, FL 32609.

This data package, identified as 423207, contained a letter from the applicant representative to EPA (dated July 28, 2014), EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (Data Matrix) 2 new studies (MRID Nos. 494585-01 and 494585-02), Statements of No Data Confidentiality Claims for all both studies, and the proposed label.

II. USE DIRECTIONS

FruitGard® granules are designed to release low levels of chlorine dioxide (ClO_2). Chlorine dioxide gas generated from FruitGard® is effective for use in controlling microbiological growth such as *Salmonella*, *E. coli,* brown rot, and others, on raw agricultural commodities such as tomatoes, melons, and potatoes during storage and shipment. The required dosages will vary depending on the items being treated.

1. Place the required amount of FruitGard® into a suitable modified reactor. A modified reactor can be the breathable sachets provided with the FruitGard®, or a plastic container (Clamshell, box, pail, etc.) with a porous cover (such as Tyvek®) that allows for the release of ClO_2 gas. For very large quantities, use of multiple reactors is recommended.
2. Add the recommended amount of acid activator material to the modified reactor containing the FruitGard® as shown below:
   a) Liquid food grade acid:
      i. Add 1 once of 50 wt% citric acid solution per 1,000 gms (2.2 lbs) of FruitGard®, or
      ii. Add 1h once of 75 wt% phosphoric acid solution per 1,000 gms (2 .2 lbs) of FruitGard®
   b) Solid Acid Impregnate: - Mix equal amounts of FruitGard® and the solid acid impregnate material *(e.g.,* Z- Series™ ZF or ZPA).
3. Mix the materials by shaking or stirring gently. FruitGard® will become active once mixed and begin releasing chlorine dioxide gas.
4. Immediately place reactor vessel / modified reactor *in* the Treatment Container holding the Raw Agricultural Produce to be treated in a way that allows gas to freely migrate across the produce.
5. Close the Treatment Container; treat for the specified time for the RAC's being treated.
6. When the fumigation is complete, unseal the space and aerate as instructed. Use ClO_2 gas detection coupons to check chlorine dioxide concentration is at or below 0.1.

III. BRIEF DESCRIPTION OF THE DATA

1. MRID 494585-01 "Efficacy of Chlorine Dioxide Gas Treatment against *Salmonella* & *E. coli* O157:H7 Inoculated onto Freshly Harvested Tomatoes & Cantaloupes" for FruitGard and Agricultural Research™ Fast Release Activator Part (B) by Dr. George Lukasik. Study conducted at BCS Laboratories, Inc. Study completion date – February 25, 2014. Project Number 1108007A/B, 1108008A/B, 110809A/B.

This study was conducted against cocktail of *Salmonella enterica* (ATCC BAA-707, BAA-709, BAA-710, BAA-711, and BAA-1673), S. Saintpaul (ATCC 9712) and cocktail of *Escherichia coli* O157;H7 (ATCC 43888, 35150, and 43895). All bacterial strains used in this study were obtained from American Type Culture Collection, with the exception of *E. coli* O157:H7 35150 and 43888 which were acquired from Microbiologics, Inc. Three batches set Precursor/Activator substrates (ZC110803TI/AZF110804TI; ZC110804LW/AZF110803TI; and ZC11041STC/AZF110330TI-B) of the product, FruitGard and Activator, were tested using EPA accepted testing protocol (April 29, 2011). The product was received ready-to-use. Fetal bovine serum was added to the culture to achieve a 5% organic soil load.

Ten (10) glass slide carriers were inoculated with 10 microliters of a suspension of test organism. The carriers were dried for no more than 40 minutes at 36.5°C until visibly dry. Carriers were treated in 10 gallon glass treatment chamber for time intervals of 5, 10, 30, 60, and 90 minutes. Following exposure, carriers were aerated for 5 minutes and transferred using sterile forceps to numbered sterile glass tubes containing 10rnL Nutrient Broth. Tubes were placed in a rack and agitated on an orbital shaker for 10-20 minutes. Following, aliquots from each tube were removed, diluted, and spread-plated onto to nutrient non-selective media (TSA), and tubes and plates were incubated at 36.5°C for 24±2 hours. Colony forming units were enumerated on plates by direct count. Tubes were checked for growth by observing turbidity in media following 24 hours and again following an additional 24 hour incubation at 36.5° C ±1.0° C. Control tubes at the beginning of the study and tubes demonstrating no growth following the 48 hour incubation were inoculated with 0.1 mL of the 10^{-6} dilution from the (+) control and verified for growth following 24±2 hours to demonstrate the absence of bacteriostatic action.

Each tomato was inoculated with the tests system as follows: three shallow surface wounds (created with a sterile scalpel blade) were inoculated with 10 µl each of the test system; five smooth surface spots were inoculated with 10 µl each; five marked spots around stem scar were inoculated with 10 µl each, and one 10 µl inoculation on the center of the stem scar. The inoculated tomatoes were allowed to dry at room temperature in a biological cabinet for up to one hour. Drying times were recorded for each study. Each tomato was assigned a number and marked with a permanent marker. Thirty-seven tomatoes were then placed into the growers/suppliers cardboard box. The location of each numbered tomato was recorded. The tomatoes were exposed to the gas treatment by seventy-five grams of each of substrate, for three hours and then the chamber was opened and aired. The control and treated tomatoes were then removed. From each tomato, the inoculated portion (upper crown that equaled about 10% of the tomato) was removed using a pre-disinfected knife. Each removed tomato section was placed in a stomacher bag containing 100 mL sterile 1% buffered peptone broth with 0.1% sodium thiosulfate. Each bag containing the inoculated portions was then, placed in a stomacher (Seward 400, GB) and blended for 2 minutes at high speed. Homogenates were then spread plated in duplicate on TSA+FAS+ST. Serial one hundred fold dilutions were made in PBS and plated as described. Plates were incubated at 36.5°C for 24 hours and the colonies on the respective plates were then enumerated. The number of colony forming units (CFU) per tomato was calculated and

reported. The percent reduction of the recovered microorganism on each treated fruit was determined based on the average number of microorganisms recovered from untreated fruit (positive control fruits). This procedure was also followed using *E. coli* O157:H7 with the exception of the emulsified tomato/buffered peptone solution being plated on TSA+Rifampicin.

Each cantaloupe was marked with a circle (2-3"), and inoculated with the test system as follows: three shallow surface wounds (created with a sterile scalpel blade) were inoculated each with 10 µl of the tests system. Additionally, 17 surface spots were inoculated with 10 µl each of the test system. Inoculated cantaloupes were allowed to dry at room temperature in the biological cabinet for up to one hour. Each cantaloupe was assigned a number and marked with a permanent marker. Thirty-seven cantaloupes were then placed into the suppliers/grower's cardboard retail boxes. The location of each numbered cantaloupe was recorded. Each box was closed and then placed into the treatment chamber. The fruits were exposed to the gaseous treatment produced by five hundred grams of each of substrate, for six hours and then the chamber was opened and aired. For each of the control and treated fruit, the inoculated marked portion was cut-off using a pre-disinfected sharp knife. The removed section was placed in a stomacher bag containing 100 mL sterile 1% buffered peptone broth with 0.1% sodium thiosulfate. Each bag was then placed in a stomacher (Seward 400, GB) and blended for 2 minutes at high speed. Homogenates were then spread plated in duplicate on TSA+FAS+ST. Serial ten-fold dilutions were also conducted and plated as described. Plates were incubated at 36°C for 24 hours and the colonies on the respective plates were then enumerated. The number of colony forming units (CFU) per cantaloupe was calculated and reported, The percent reduction of the recovered microorganism on each treated fruit was determined based on the average number of microorganisms recovered from untreated fruit (positive control fruits). This procedure was also followed using *E. coli* O157:H7 with the exception of the emulsified cantaloupe/buffered peptone solution being plated on TSA+Rifampicin.

Inoculated cantaloupes with three-strain cocktail *E. coli* O157:H7, (ATCC 35150, 43895, and 43888), were tumbled rapidly in the aqueous bath of 200 ppm free chlorine washes solution, for three minutes. They were then removed, the inoculated areas cut off, and each was placed in a stomacher bag containing 100 ml sterile 1% peptone broth with 0.1% sodium thiosulfate. Each of the bags containing the cantaloupe section was placed in a stomacher (Seward 400) and blended for 2 minutes at high speed. Homogeriates were then spread plated in duplicate on TSA+FAS+ST, Plates were incubated at 36"C for 24 hours and the colonies on the respective plates were enumerated. The number of CPU per fruit was calculated.

V. RESULTS

Percent of Total Chlorine Dioxide Released

Time Total (Min)	% ClO_2 Released per Total Time	Time Interval (Min)	% ClO_2 Released per Time Interval
0 to 5	12%	0 to 5	12%
0 to 10	24%	5 to 10	12%
0 to 60	48%	10 to 60	24%
0 to 90	62%	60 to 90	14%
0 to 180	87%	90 to 180	25%
0 to 360	100%	180 to 360	13%

Glass Carrier Contact Time Study

- At a 5-minute contact time, efficacy was less than the Agency's required performance criteria (99.9013% for *Salmonella* and 99.8752% for *E. coli* O157:H7. Of the 20 carriers used in the study, it was found that the 8 carriers positioned closest to the product media in the test chamber exhibited no growth; however, the 12 carriers positioned farthest from the product media in the test chambers exhibited growth.
- At contact times of 10-90 minutes, efficacy exceeded 99.9999%. The amount of FruitGard™ used in this study was equal to the dose applied in the tomato study. The age of the test material did not affect the results.

Tomato Exposure Study

- At a 1-hour activation and exposure time, efficacy was less than the Agency's required performance criteria (99.6%).
- At a 3-hour activation and exposure time, efficacy exceeded 99.9% for *Salmonella* and *E. coli* O157:H7. The amount of FruitGard™ used in this study, was 75 grams, or 6.6 grams of FruitGard™ media per Kg fruit; per Figure 1, this equates to 47 mg ClO2 per Kg fruit produced over the 3-hour exposure period. The age of the test material did not affect the study results.

Cantaloupe Exposure Study

- At a 3-hour exposure time, efficacy was less than the Agency's required performance criteria (99.6%).
- At a 6-hour exposure time, efficacy exceeded 99.9% for Salmonella and E. coli O157:H7. The amount of FruitGard™ used in this study was 100 grams, or 12.8 grams of FruitGard™ media per Kg fruit; per Figure 1, this equates to 105 mg ClO2 per Kg fruit produced over the 6-hour exposure period. The age of the test material did not affect the study results.

Chlorine Wash on Cantaloupes Study

- These washes yielded an average percentage reduction/efficacy control of 97.4% on *E. coli* O157:H7 for 3 minutes contact time at 200 ppm free chlorine solution.

VI. CONCLUSION

1. Based on the submitted data (MRID 494585-01)

- The Glass Carrier study used the same amount of FruitGard™ applied in the tomato study and established a minimum contact time of 10 minutes with greater than a six log (99.9999%) reduction of both *Salmonella* and *E. coli* O157:H7.
- At a FruitGard™ dose of 6.6 gm per Kg fruit and a 3-hour activation and exposure time for tomatoes, efficacy exceeded 99.9% for the target organisms. Thus, 6.6 gm/kg fruit and 3-hours is the recommended dose, activation and exposure time for use of FruitGard™ on tomatoes.
- At a FruitGard™ dose of 12.8 gm per kg fruit and a 6-hour activation and exposure time for cantaloupes, efficacy exceeded 99.9% for the target organisms. Thus, 12.8 gm/kg fruit and 6-hours is the recommended dose, activation and exposure time for the use of FruitGard™ on cantaloupes.
- When used 200 ppm chlorine washes against the target organisms for 3 minutes contact time, percentage reductions were between 97.0 - 97.8%.

- However, minimum air concentration of ClO_2 necessary for effectiveness of treatment and maximum volume of treatments were not explored, reported, or disclosed.

2. Potential microorganisms trapped between touching surfaces of produce when piled up or put in a container (crate, boxes, bags) were not tested. This could be done by simply dipping tested Tomatoes and Cantaloupes in adjusted inoculum solutions.

3. Produce must be treated separated from each other and from any other surface by a material (mesh, porous cellulose, breathable materials) that will allow flow of chlorine dioxide to all surfaces of produce.

4. If treatment can be done in storage rooms (possibly warehouse), volume of rooms and air concentrations of ClO_2 must be taken into consideration when treating. Maximum volume of treatment for each proposed treatment must be tested and stated on the label.

5. **Submitted efficacy data (MRID 494585-01) do not support label claims. Tests must be re-conducted to include necessary parameters/conditions detailed above.**

VII. LABEL

1. **Fungicide/Virucide/Sporicide** claims on the label must be deleted. Data were not generated to support those claims.

2. Potatoes' treatment claims of 1 gm/kg for 6 hours treatment time are for fumigation against non-pathogenic spoilage organisms, such as yeasts and molds. These claims must not be mixed with tomatoes and cantaloupes claims against *Salmonella* and *E. coli* O157:H7. Registrant must clearly separate these claims on label.

3. **Label claims against *Salmonella* and *E. coli* O157:H7 are not supported by submitted data**. Produce must be separated from each other and from any other surface by a material (mesh, porous cellulose, breathable materials) that will allow flow of chlorine dioxide to all surfaces of produce until microorganisms trapped between touching produce is investigated. Maximum volume of each treatment must be tested.